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14. **ABSTRACT** We have developed a mouse model for ovarian cancer that allows monitoring of tumor-specific T cell clones as they encounter ovarian tumors in vivo. We "tagged" the neu oncogene with two defined T cell epitopes so as to confer recognition by available T cell receptor (TCR) transgenic T cells. When expressed in the murine ovarian tumor cell line ID8, epitope-tagged neu (designated neuOT1/OT2) induces the formation of aggressive ovarian adenocarcinomas that express the epitope tags and hence are recognizable by adoptively transferred TCR trangenic T cells. We successfully made the neuOT1/OT2expression construct and stably expressed it in an aggressive subclone of the ID8 cell line, designated ID8-G7, which was derived by serial in vivo passage of the original ID8 line. When injected intraperitoneally into syngenic mice, ID8-G7 cells expressing neuOT1/OT-II give rise within one month to disseminated ovarian cancer with extensive ascites (Aim 1). CD8+ (OT-I) T cells specific for neuOT1/OT-II proliferate extensively after adoptive transfer into tumour-bearing hosts and, remarkably, induce complete tumour regression within 10 days in a dose-dependent manner (Aim 2). In the next year, we will test whether the dose-dependency of this response can be mitigated by use of autoimmune-prone Cbl-b-deficient CD8+ T cells (Aim 3).

15. SUBJECT TERMS
Tumor immunology, immunotherapy, animal models, CD4+ and CD8+ T 15. Number of Pages (count all pages including appendices) cells, HER2/neu, tumor antigens

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DAMD17-01-1-0733 Annual Progress Report 2005

PI: Brad H. Nelson, Ph.D.

Title of Project: Eliciting Autoimmunity to Ovarian Tumors in Mice by Genetic Disruption of T Cell Tolerance Mechanisms

Introduction:

Research in the fields of basic immunology and autoimmunity has identified several distinct mechanisms through which immune tolerance is established and maintained in the normal host, and additional mechanisms will likely be identified in future. We hypothesize that ovarian tumors are recognized in an antigen-specific manner by T cells but induce immunologic tolerance through one or more of these homeostatic mechanisms, which have evolved to protect the host from autoimmune attack. We further hypothesize that tolerance to ovarian tumors can be overcome by disrupting critical components of tolerogenic pathways through genetic manipulation of T cells. To test this hypothesis, we proposed to develop a murine model for ovarian cancer that will allow, for the first time, precise monitoring of the functional responses of naïve, tumor-specific CD4+ and CD8+ T cell clones to ovarian tumors. Multiple properties of tumor-reactive T cells will be assessed *in vivo*, including their localization, activation, anergic status, proliferation and apoptosis. Differential responses and anti-tumor activities of the CD4+ and CD8+ T cell subsets will be investigated. Finally, the model will be used to evaluate the functional responses of tumor-specific CD4+ and CD8+ T cells that are genetically pre-disposed to autoimmune activity. The first tolerogenic pathway tested will be that involving the Cbl-b gene, as T cells lacking Cbl-b have a greatly reduced requirement for CD28 co-stimulation and demonstrate hyperactivity in vivo with profound autoimmune sequelae. The specific aims of this proposal are:

Aim 1. To generate an ovarian tumor cell line that is recognized by antigen-specific CD4+ and CD8+ T cell clones from TCR transgenic mice.
Aim 2. To define the mechanisms by which ID8 ovarian tumors evade rejection by tumor-specific CD4+ and CD8+ T cells.
Aim 3. To determine whether tumor-specific CD4+ and CD8+ T cells lacking the Cbl-b gene show enhanced functional responses to ovarian tumors.

Body:

Aim 1: To generate an ovarian tumor cell line that is recognized by antigen-specific CD4+ and CD8+ T cell clones from TCR transgenic mice.

As described in the previous progress report, we had to generate a more aggressive ovarian tumor cell line for our experiments, as we encountered problems with spontaneous rejection of the original ID8 cell line when it was made to express epitope-tagged *neu* ($neu^{OT\text{-}I/OT\text{-}II}$). We generated a more aggressive subclone by serial transplantation of ID8 cells in syngeneic host mice. The new subclone (ID8-G7) induces tumors in just 30-40 days, as opposed to the 120-day latency of the original ID8 cell line. We have successfully transfected the new subclone with the $neu^{OT\text{-}I/OT\text{-}II}$ construct under the control of the β-actin promoter (ID8-NOO) and achieved stable expression. However, the transfected cells are still rejected by wild-type C57/Bl6 hosts due to expression of the highly immunogenic OT-I and OT-II epitopes. Fortunately, for another DOD-funded project, we created transgenic mice that express $neu^{OT\text{-}I/OT\text{-}II}$ in mammary epithelium under the control of the MMTV promoter (MMTV/NTOO mice). These mice are

tolerant to *neu*$^{OT\text{-}I/OT\text{-}II}$ as it is essentially a self protein. MMTV/NTOO mice do not develop mammary tumours until >12 months of age, therefore they can serve as hosts for ID8-G7-induced ovarian tumours at younger ages. Indeed, when MMTV/NTOO mice are injected i.p. with the ID8-G7/*neu*$^{OT\text{-}I/OT\text{-}II}$ cell line, disseminated ovarian cancer forms within one month in 100% of cases and is associated with extensive ascites. Thus, we now have a fully operational ovarian cancer model involving epitope-tagged *neu*.

Aim 2: To define the mechanisms by which ID8 ovarian tumors evade rejection by tumor-specific CD4+ and CD8+ T cells.

We obtained from other labs CD8+ and CD4+ T cells expressing TCR transgenes specific for the OT-I and OT-II epitopes on *neu*$^{OT\text{-}I/OT\text{-}II}$, respectively. To evaluate the CD8+ OT-I T cell response to ovarian tumors in our model, we adoptively transferred 10 million lymphocytes from TCR transgenic OT-I donor mice into MMTV/NTOO transgenic mice bearing established ovarian tumors. Control mice received a similar dose of CD8+ T cells expressing an irrelevant TCR (obtained from P14 donor mice). The OT-I T cells (but not the negative control P14 T cells) began to proliferate within 3 days of adoptive transfer and, by Day 9 constituted as much as 65% of the circulating CD8+ T cell population in blood and 96% of CD8+ T cells in ascites (Figure 1 and data not shown). Whereas all control mice (6/6) receiving P14 T cells had to be euthanized by day 10 after adoptive transfer due to progressive tumour growth, all mice (6/6) receiving OT-I T cells achieved complete tumor regression and fully recovered by day 10 post-adoptive transfer. Recurrences have been observed in all animals, typically commencing ~30 days after adoptive transfer, a period in which OT-I T cells are still abundant in peripheral blood. Some recurrent tumours no longer express *neu*$^{OT\text{-}I/OT\text{-}II}$, whereas others remain positive for *neu*$^{OT\text{-}I/OT\text{-}II}$ and MHC Class I, suggesting ID8 tumours can escape CD8+ T cells by more than one means.

We have reduced the transferred cell dose to one million lymphocytes and still observed the activation and proliferation of donor T cells and infiltration of the tumor site (Figure 2). However, tumor regressions are never observed at lower T cell doses. In principle, if 1 x 10^6 T cells were to undergo only 3 additional rounds of proliferation, they could theoretically reach equivalent numbers as achieved with the 1 x 10^7 dose. This leads us to question what factors may limit the expansion or persistence of CD8+ T cells in the ovarian tumour microenvironment.

In this regard, we observed that activated OT-I T cells express CD25 (the alpha subunit of the IL-2 receptor) when they infiltrate ovarian tumors, yet are CD25-negative in blood, lymph node and ascites (Figure 3). This suggests that OT-I cells may undergo local, IL-2-induced proliferation within the tumour bed, which may be an important determinant of the final population size. Indeed, OT-I T cells rendered genetically deficient for the IL-2 receptor beta subunit show reduced numbers in ascites after adoptive transfer into tumour-bearing hosts (Figure 4). This indicates IL-2 signaling may play an important role in sustaining antigen-specific T cell proliferation and tumor cell killing. Consistent with this, we find that tumour-infiltrating antigen-specific T cells have undergone more cell divisions than OT-I cells residing in lymph notes (Figure 5). Thus, future studies will investigate the relationship between the extent of T cell proliferation in the tumour microenvironment and tumour regression.

Aim 3: To determine whether tumor-specific CD4+ and CD8+ T cells lacking the Cbl-b gene show enhanced functional responses to ovarian tumors.

As described in last year's progress report, the Cbl-b -/- mice we received from Dr. Josef Penninger's lab were not on a pure B6 background. Therefore, we have had to backcross the

mice onto the B6 background. We have completed a 9th generation of backcrossing, so these mice are now ready for experiments on the C57Bl6 background. Breeding of OT-I, OT-II, TEa and P14 TCR transgenes onto the Cbl-b background is underway.

Key Research Accomplishments:

The following items have been completed or are underway:

Task 1. To generate an ovarian tumor cell line that is recognized by antigen-specific CD4+ and CD8+ T cell clones from TCR transgenic mice (July 2003-Dec 2003).

a. Evaluate signaling and transforming properties of epitope-tagged and untagged version of *neu* in cell lines; if problems noted, modify epitopes as needed. ***completed***

b. Generate ID8 cell subclones that stably express $neu^{OT\text{-}I/OT\text{-}II}$; perform in vitro assays to evaluate recognition of OT-I and OT-II epitopes by CD4+ and CD8+ T cells from TCR-transgenic mice. ***completed***

c. Inject ID8/$neu^{OT\text{-}I/OT\text{-}II}$ cells intraperitoneally into MMTV/NTOO transgenic mice to establish ovarian cancer (July 2003-Dec2003). ***completed***

Task 2. To define the mechanisms by which ID8 ovarian tumors evade rejection by tumor-specific CD4+ and CD8+ T cells (Jan 2004-July 2005).

a. Generate sufficient numbers of mice bearing tumors expressing $neu^{OT\text{-}I/OT\text{-}II}$. ***completed***

b. Perform immunological studies of adoptively transferred OT-I- and OT-II-specific T cells and control T cells in mice bearing ovarian tumors expressing $neu^{OT\text{-}I/OT\text{-}II}$, as per Aim 2. ***in progress***

Task 3. To determine whether tumor-specific CD4+ and CD8+ T cells lacking the Cbl-b gene show enhanced functional responses to ovarian tumors (July 2005-June 2006).

a. Breed OT-I, OT-II, TEa and P14 TCR transgenes onto the Cbl-b background (Months 1-12). ***breeding is underway***

b. Generate sufficient numbers of mice bearing tumors expressing $neu^{OT\text{-}I/OT\text{-}II}$ (Months 1-12). ***completed***

c. Perform immunological studies of adoptively transferred Cbl-b-deficient OT-I- and OT-II-specific T cells and control T cells in mice bearing ovarian tumors expressing $neu^{OT\text{-}I/OT\text{-}II}$ (Months 4-12). ***commencing Nov. 2005***

Reportable Outcomes:

Conference proceedings:

Proliferation and Differentiation of CD8+ T Cells in the Absence of IL-2/IL-15 Receptor beta Chain Expression or STAT5 Activation. Ryan M. Teague, Richard M. Tempero, Sunil Thomas, Murali-Krishna Kaja and **Brad H. Nelson**. Annual Meeting of the American Association for Cancer Research, Orlando, March 2004.

Proliferation and Differentiation of CD8+ T Cells in the Absence of IL-2/IL-15 Receptor beta Chain Expression or STAT5 Activation. Ryan M. Teague, Richard M. Tempero, Sunil Thomas, Murali-Krishna Kaja and **Brad H. Nelson**. 12th International Congress of Immunology and 4th Annual Conference of the Federation of Clinical Immunology Societies (FOCIS), Montreal, July 2004, Publication Number: (76PM) W8.29

Mapping and Manipulating the Immune Response to Ovarian Cancer. **Brad H. Nelson**, Brad C. Stone and Cassian Yee. Second Canadian Conference on Ovarian Cancer Research, Ottawa, May 2004.

Monitoring the T cell response to spontaneous mammary tumors using a novel transgenic mouse model. Erika M. Wall, Katy Milne, **Brad H. Nelson**. Annual Meeting of the American Association for Cancer Research, Anaheim, April 2005.

Monitoring the T cell response to spontaneous mammary tumors using a novel transgenic mouse model. Erika M. Wall, Katy Milne, **Brad H. Nelson**. Canadian Society for Immunology Annual Meeting, Whistler BC, April 2005.

Regression of Advanced Ovarian Cancers After Adoptive Transfer of CD8+ T Cells in a Novel Murine Model. Taimei Yang, Erika M. Wall, Katy Milne and **Brad H. Nelson.** NCI/SPORE Annual Meeting, Washington DC July 2005.

Evaluation of the T Cell Response to Mammary Tumours Using a Novel Transgenic Mouse Model. Elaine K. Wong, Erika M. Wall, Katy Milne and **Brad H. Nelson**. Translational Research in Radiation Oncology Symposium, San Francisco, CA, August 2005.

Invited presentations:

Mapping and Manipulating the Immune Response to Ovarian Cancer. **Brad H. Nelson**, Brad C. Stone and Cassian Yee. Second Canadian Conference on Ovarian Cancer Research, Ottawa, May 2004.

Mapping and Manipulating the Immune Response to Cancer. **Brad H. Nelson**, Canada's Michael Smith Genome Sciences Centre, Vancouver, Sept. 2004.

The Immune Response to Ovarian Cancer. **Brad H. Nelson**. National Ovarian Cancer Association, Educational Session, Victoria BC, Nov. 20, 2004.

The Immune Response to Ovarian Cancer. **Brad H. Nelson**. BC Cancer Agency's Annual Meeting, Pharmacy Session, Vancouver, Nov. 2004.

Mapping and Manipulating the Immune Response to Cancer. **Brad H. Nelson**. University of Victoria, Feb. 4 2005.

The Immune Response to Ovarian Cancer. **Brad H. Nelson**. OBGYN Grand Rounds, Vancouver General Hospital, Feb.16 2005.

Mapping and Manipulating the Immune Response to Cancer. **Brad H. Nelson**. Canadian Association of Medical Oncologists Annual Meeting, Montreal, March 2005.

Tracking and Manipulating the Immune Response to Cancer. **Brad H. Nelson**. McMaster University, Hamilton Ontario, Oct. 2005.

Career advancement:

The PI, Brad Nelson, has been appointed Director of the Research Laboratories for the British Columbia Cancer Agency's Vancouver Island Centre (Victoria, BC). His work in this animal tumor model was integral to his success in this competition. He moved there on July 1, 2003. (The DOD has already been informed of this move.)

Conclusions:

The mouse model we have developed should lead to an improved understanding of the immune response to ovarian cancer and may facilitate the development of novel immune-based therapies or immunopreventive strategies for this disease. Toward this goal, we have now created a dually epitope-tagged version of *neu* that is recognized by the appropriate CD4+ and CD8+ T cells. We have successfully established ovarian cancer in MMTV/NTOO transgenic mice. Adoptive T cell studies have shown that CD8+ OT-I T cells alone are able to induce tumor regression in a dose-dependent manner. We are now investigating the role of IL-2-induced T cell proliferation in tumour regression. Finally, we have obtained and are currently breeding the Cbl-b -/- mice required for Aim 3. No other changes to the research plan are expected.

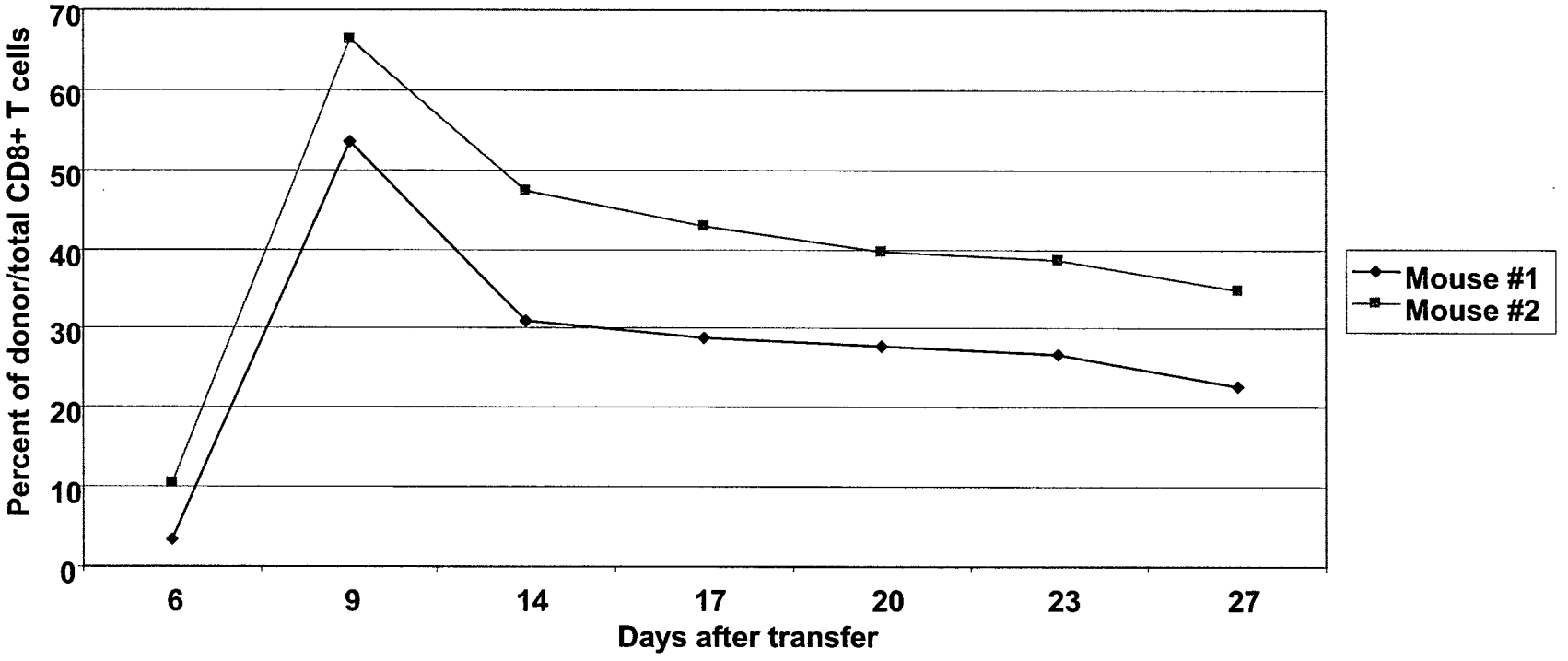
References:

None.

Appendices:

See accompanying Figures 1-5.

Fig.1. Expansion of adoptively transferred CD8+ OT-I T cells in peripheral blood in response to *neu*$^{OT\text{-}I/OT\text{-}II}$ expressing ovarian tumours in MMTV/MTOO mice.



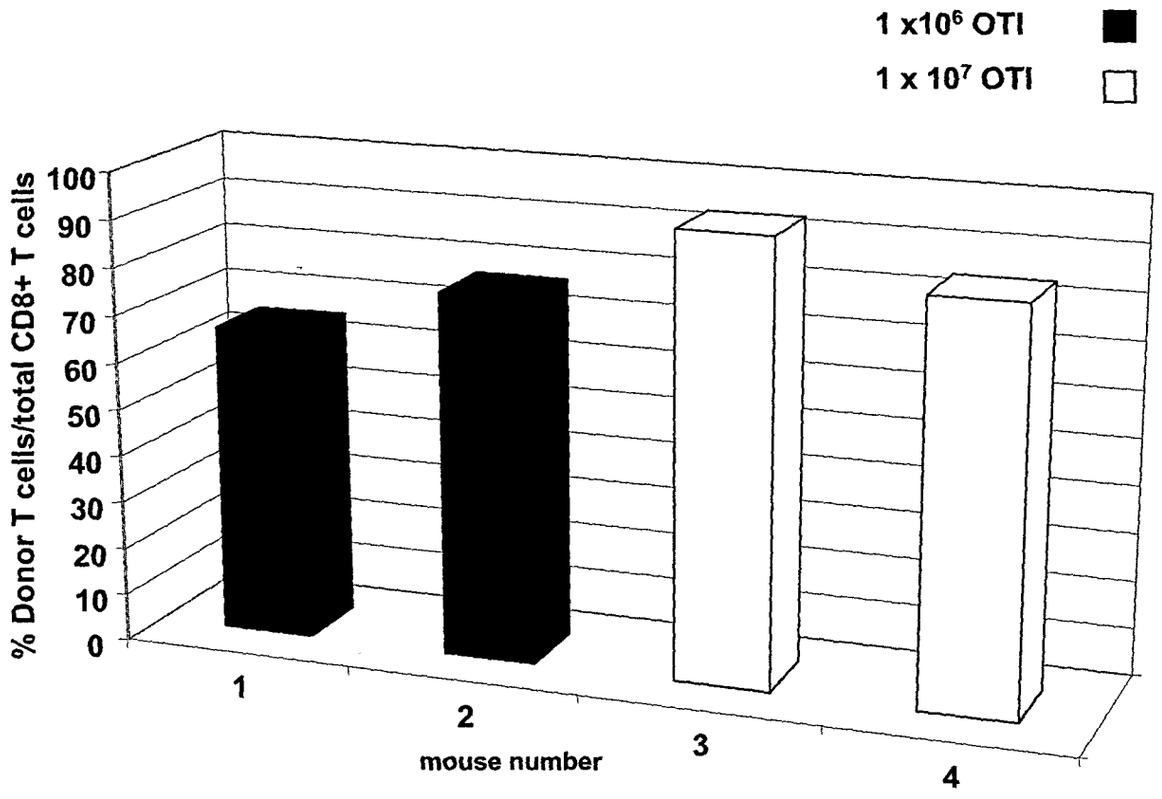


Fig. 2. Percentage of tumour-infiltrating OT-I T cells relative to total CD8+ T cells after adoptive transfer of 1 x 10^6 versus 1 x 10^7 lymphocytes from OT-I transgenic mice into ovarian tumor-bearing hosts.

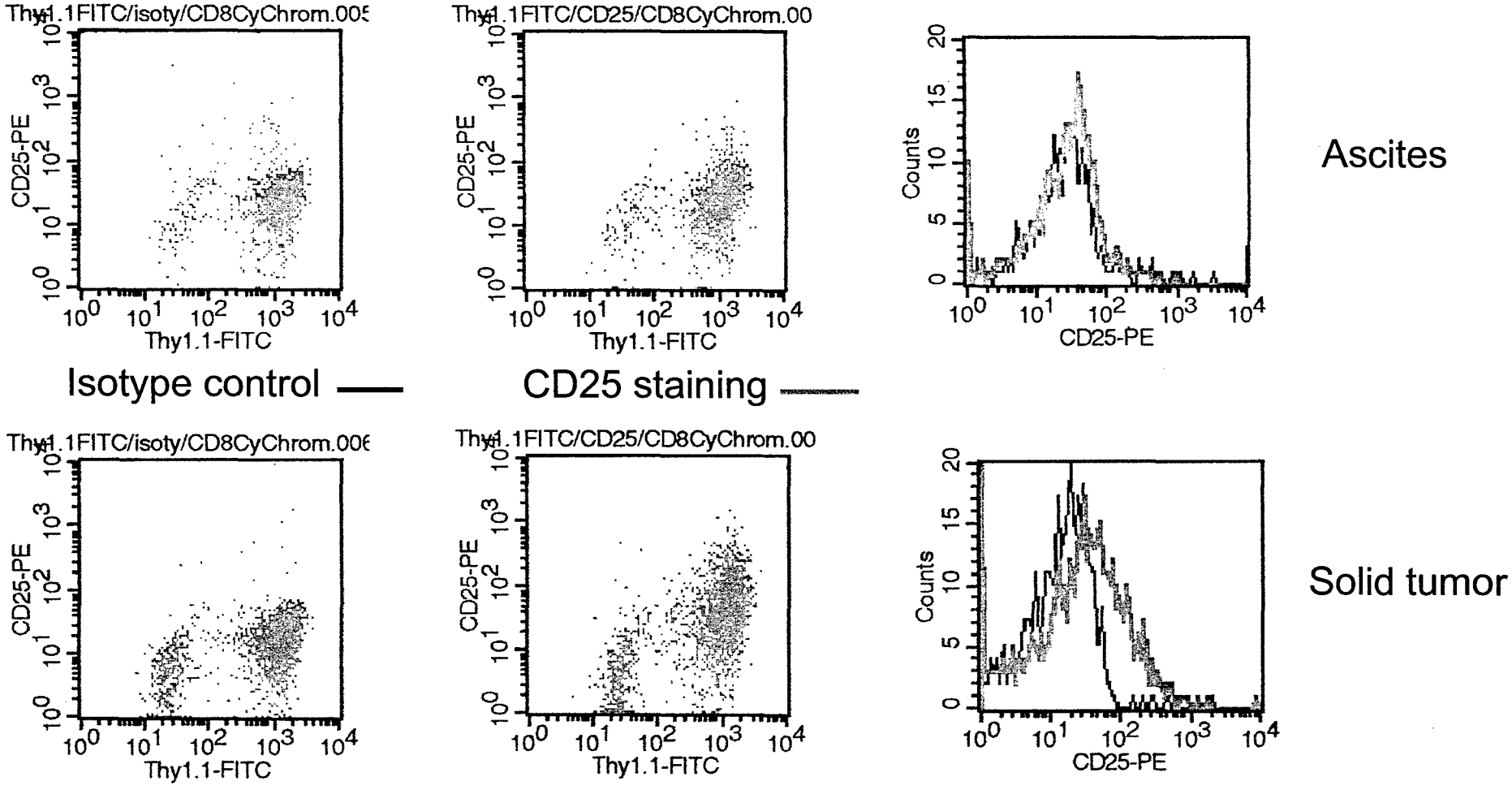


Fig. 3. CD25 expression on OT-I donor T cells in ascites and solid tumor after adoptive transfer into ovarian tumour-bearing mice.

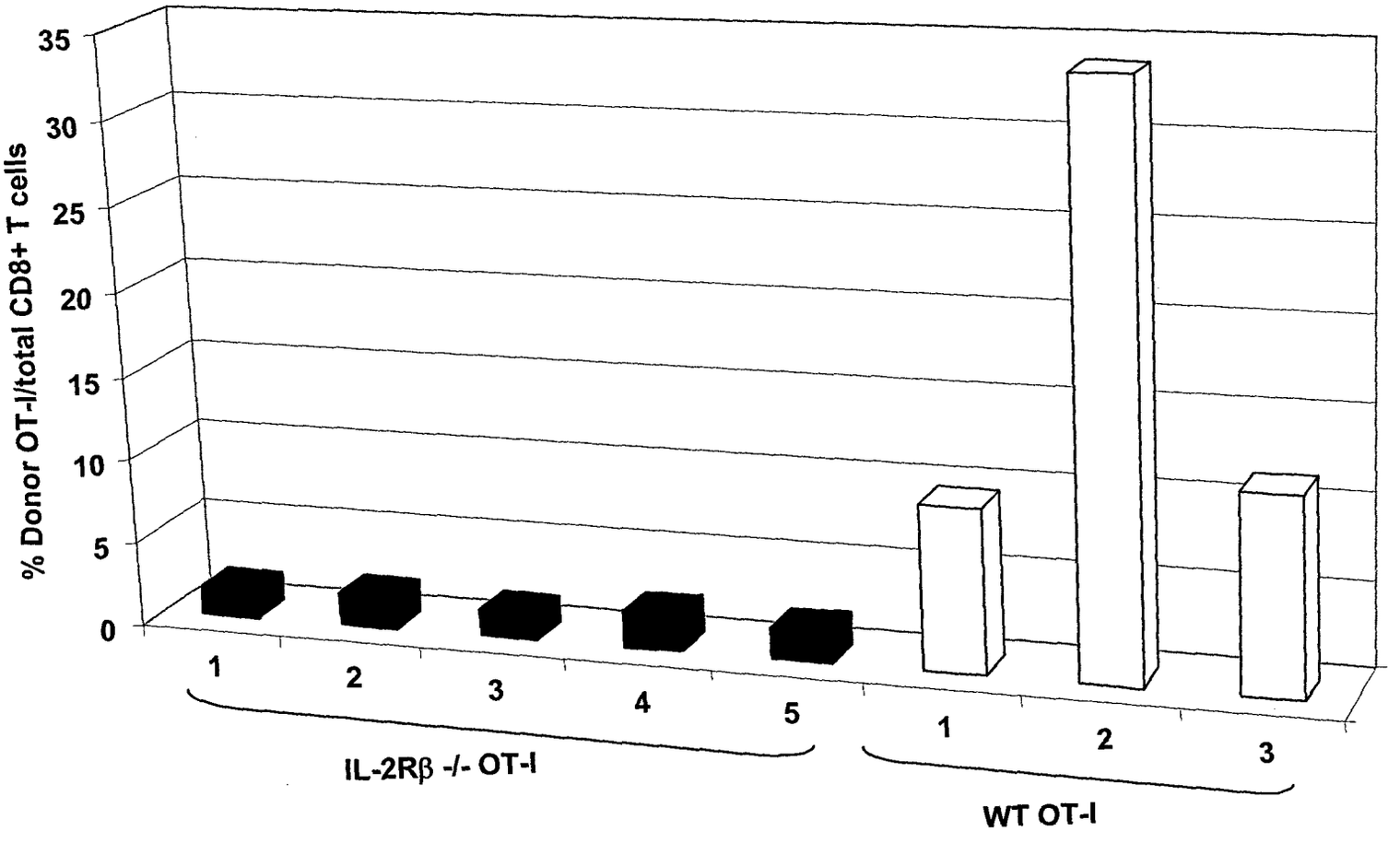
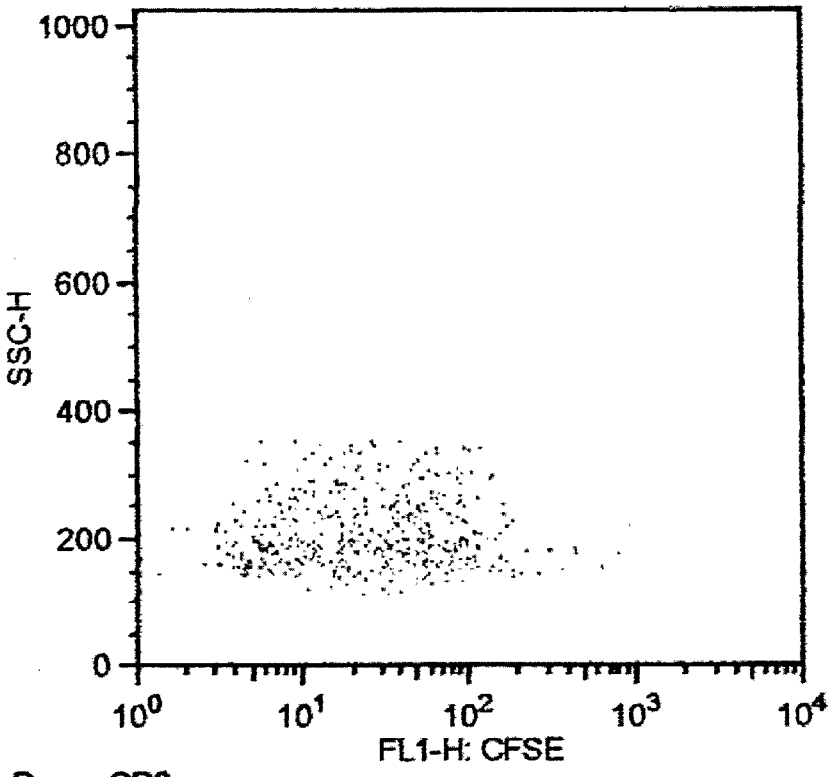
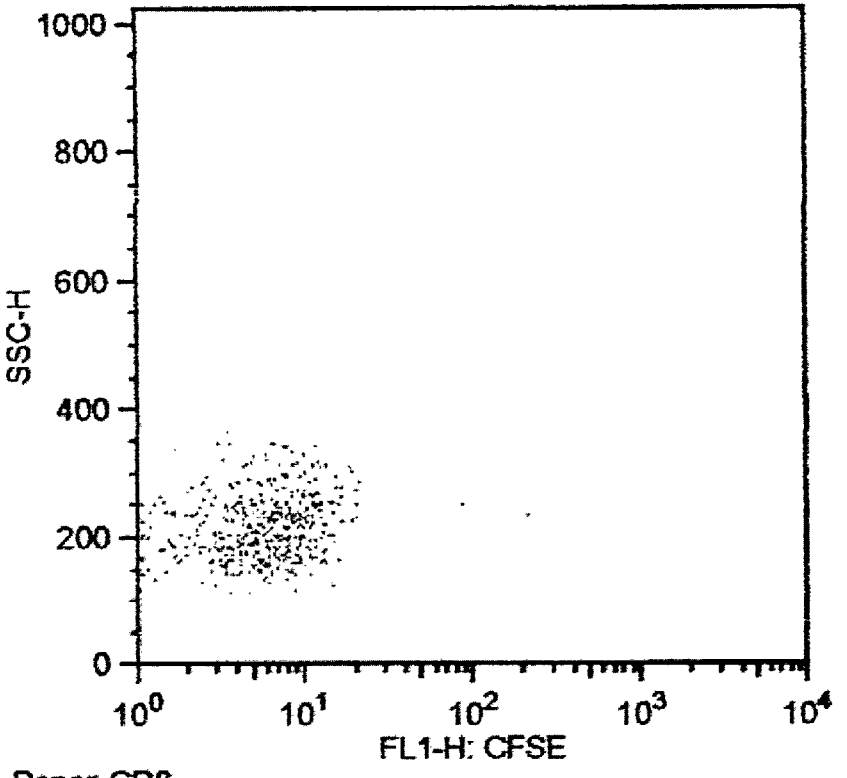


Fig. 4. CD8+ OT-I T cells deficient in the IL-2 receptor beta subunit (an essential component of the IL-2 and IL-15 receptors) show reduced accumulation in the ascites of ovarian tumour-bearing mice (Day 7 after adoptive transfer).





Lymph notes

Solid tumor

Fig. 5. Adoptively transferred OT-I T cells infiltrating ovarian tumours have undergone more cell divisions than those in draining lymph nodes, as indicated by lower CFSE content.